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Life history of the Levant water frog, *Pelophylax bedriagae* (Amphibia: Anura: Ranidae) in western Iran

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Abstract

In the present study, we studied the development and morphology of different larval stages of *Pelophylax bedriagae* (Camerano) at two rearing temperatures (20 and 24 °C). Eggs were collected from a breeding site in western Iran. Diagnostic morphological characters are provided for Gosner (1960) larval stages 1–46. The larvae hatched about seven days after egg deposition in the laboratory. A principal diagnostic feature, the formation of the funnel-shaped oral disc, became discernible about ten days after hatching at Gosner stage 21 and degenerated at Gosner stage 42. Based on our results, the longest metamorphosis time was observed at 20 °C whilst the shortest metamorphosis time occurred at 24 °C. The largest body length of larval *P. bedriagae* measured about 54 mm in 70 days after egg deposition. Compared with the majority of other Palearctic anurans, it appears that embryonic and larval development is usually slow in *P. bedriagae*.

Key words: Development, larval stages, *Pelophylax bedriagae*, temperatures

Introduction

Amphibians are unique among the tetrapods in the complexity of their life cycles and the degree to which their life cycles vary in response to environmental conditions (Wells, 2007). The descriptions of anuran embryos and larvae are indispensable to the study of frog taxonomy, phylogeny, life history, and behavior (Gosner, 1960). Globally, amphibians are declining faster than any other vertebrate group (Stuart et al., 2004). Populations of ectothermic animals have a strong dependence on ambient temperature because they do not have an efficient mechanism for physiological thermoregulation (Brattstrom, 1963). Temperature affects amphibian larval developmental rates directly (Hayes and Licht, 1993).

The Family Ranidae is distributed in the whole of Iran except desert areas and has two genera and three species in Iran (Rastegar-Pouyani et al., 2008; Bashiri et al., 2015). The Levant water frog, *Pelophylax bedriagae* (Camerano), is distributed across the Eastern Mediterranean (Frost, 2011). The species is widespread along the western and south-western parts of Iran (Pesarakloo et al., 2017). *Pelophylax bedriagae* is threatened by habitat loss as a consequence of wetland drainage, pollution, drought and urbanization of coastal areas (AmphibiaWeb, 2019).

In this study, first, the external form of the embryonic stages of the Levant water frog, *P. bedriagae*, are described from fertilization to metamorphosis. Then additional comparisons are made here between the developmental larval of *P. bedriagae* and those of other species of Anura with similar larval development. In addition, we present the first staging table for *P. bedriagae* or probably for any *Pelophylax* species.

Material and Methods

Two egg clutches of *Pelophylax bedriagae* were collected from a natural and clean pond in the Khorramabad region of western Iran, at 33°12'N, 48°53'E and 2500 m above sea level (a.s.l.), on 19 April 2017. Clutches were collected by hand and stored in separate polyethylene tubs (600 mm × 400 mm × 250 mm) filled with water from the natural habitat and transported to the Biology Laboratory, Arak University, Arak, Iran.

The experiment involved two rearing temperatures: 1) 20 °C and 2) 24 °C because our observation had shown that, they reflected the average lower and upper estimates of temperatures that *P. bedriagae* tadpoles experience in Khorramabad systems during the period between April and July. Tadpoles hatched from eggs 1 day after collection, and once tadpoles had hatched the egg jelly was removed from the tubs. The experimental conditions provided were based on Pesarakloo et al. (2015). The hatched tadpoles were housed in plastic rearing tanks (600 mm × 400 mm × 250 mm), and an aquarium water heater (50 W) was placed in the plastic rearing tanks to set the experimental treatment temperature; each plastic rearing tank had 300 tadpoles and 40 L water. Experimental samples were kept in a temperature and light-controlled room maintained at 18 ± 2 °C ambient temperature and a 12 h–12 h light–dark period.

To ensure that temperatures were maintained at treatment temperatures throughout the entire experimental period, water temperatures were monitored on a weekly basis using a calibrated digital thermometer probe (Pesarakloo et al., 2015). Water volume adjustments were carried out on a weekly basis, and partial water changes (∼30%) were made once per week. Animals were exposed to a constant feeding regimen throughout the entire experimental period. The food consisted of a mixture of boiled spinach, lettuce and potato (Pesaraklou et al., 2008).

To study the morphological changes on the specimens at various times tadpoles were fixed in 4% formalin. Upon the appearance of hindlimbs and lung ventilation, we placed a piece of stone inside rearing tanks with apart of the stone out of the water. After completing metamorphosis, the fixed samples were measured with a digital caliper and photographed with a Stereomicroscope equipped with a digital camera. The provided pictures of the development stages of *P. bedriagae* were compared with the development and metamorphosis of *Bufo valliceps* (Gosner, 1960).

Results

The number of eggs in two egg clutches varied from 5000 to 6000, and the embryonic stages include: cleavage, gastrulation, neurulation, elongation and etc. observed in this study. For detailed staging of the following early developmental stages see Table 1.

The larvae hatched about seven days after egg deposition with the yolk reservoir clearly visible. Between 600 and 650 larvae hatched per egg clutches. About five days after hatching, the tadpoles remained clustered in close groups on the bottom. For detailed staging of the following early developmental stages see Table 2.

Table 1: Developmental stages of *Pelophylax bedriagae*, from stage 1–17; stage diagnostic characters according to Gosner (1960).

Stage number	Age (days)	Diagnostic features
1	1	Fertilization
7	1	32 cells
9	1	Late cleavage
10	2	Dorsal lip
11	2	Yolk plug
12	3	Late gastrula
14	4	Neural fold
15	4	Elongation, Rotation
16	4	Neural tube, Gill plate
17	5	Tail bud, Adhesive gland

Table 2: Developmental stages of *Pelophylax bedriagae*, from stage 18–22; stage diagnostic characters according to Gosner (1960).

Stage number	Age (days)	Diagnostic features
18	10	Muscular response to water movement; eye region begins to develop
19	15	Heart beat visible; eye pigmentation distinctly discernible; oral region begins to stretch upwards; developing dark pigmentation on body dorsum and tail; yolk reservoir reduced and blood vessels discernible
20	19	Development and circulation of external gills; elongated oral region; last stage with distinctly visible yolk reservoir; tail longer than body
21	23	Cornea transparent; funnel mouth discernible; dark body and tail musculature with transparent and distinctly developed fin
22	-	Fin circulation begins; dark dorsal pigmentation brightens

The funnel mouth became discernible about 10 days after hatching. About three days later, the larvae began to move to the water surface, and after about 20 days post-hatching all tadpoles were feeding. Forty five days after hatching the tadpoles had reached lengths of up to 26 mm. For detailed staging of the following advanced developmental stages see Table 3.

On average around 70 days after hatching, at Gosner stage 27, hindlimbs started to develop. At this time, the largest tadpoles measured about 54 mm. Shortly before metamorphosis the funnel mouth was reduced. About 111 days after egg deposition the first larvae completed metamorphosis. At that time the metamorphosed had total body lengths of 16–19 mm. Reabsorption of the tail took three to five days. While most of tadpoles in the 24 °C treatment had finished their development and commenced with metamorphosis after 111 days, some individuals in the 20 °C treatment showed a distinctly slower developmental progress which took up to 140 days, or longer in some cases.

For the developmental Gosner stages 1–17 (see Table 1 and Figs. 1A-D and 2A-D), 18–22 (see Table 2 and Fig. 2E-I), and 27–46 (see Table 3 and Figs. 3A-E, 4 and 5A-D), we assessed diagnostic morphological features and age in days based on 3–4 individuals. The tadpoles in all stages possess a labial tooth row formula of 2(1)3/(1). This characteristic became discernible about ten days post-hatching (Fig. 2I).

Table 3: Developmental stages of *Pelophylax bedriagae*, from stage 27–46; stage diagnostic characters according to Gosner (1960).

Stage number	Age (days)	Diagnostic features
27	71	Hindlimbs bud visible; length of hindlimbs > 0.5 × basal width
28	73	Length of hindlimbs > basal width; length of hindlimbs < length of vent tube
29	76	Length of hindlimbs > 1.5 × basal width
30	79	Length of hindlimbs = 2 × basal width; length of hindlimbs = length of vent tube
31	81	Foot paddle-shaped
32	83	Indentation between 4th and 5th toe
33	85	Indentation between 3rd and 4th toe
34	87	Indentation between 2nd and 3rd toe
35	89	Indentation of all toes; hindlimb > vent tube
36	90	Toes 3-5 separated
37	93	All toes separated; pigmentation of hindlimbs darkens
38	95	Metatarsal tubercle visible
39	97	Subarticular patches slightly visible
41	99	Funnel mouth atrophy; vent tube gone
42	101	Funnel mouth degenerated; forelimbs emerged; spiracle opening disappeared; Mouth beneath nostril
43	103	Snout pointed; eyeballs starting to protrude; mouth between nostril and eye
44	106	Terrestrial life modus; tail atrophy; eyeballs further pointed; longitudinal ridges on back; mouth beneath eye
45	109	Tail mostly reduced; mouth posterior to eye
46	111	Change of pigmentation (cream, fawn); lappet of snout and eyeballs visible; ridges on back and head become more distinct; tail completely resorbed

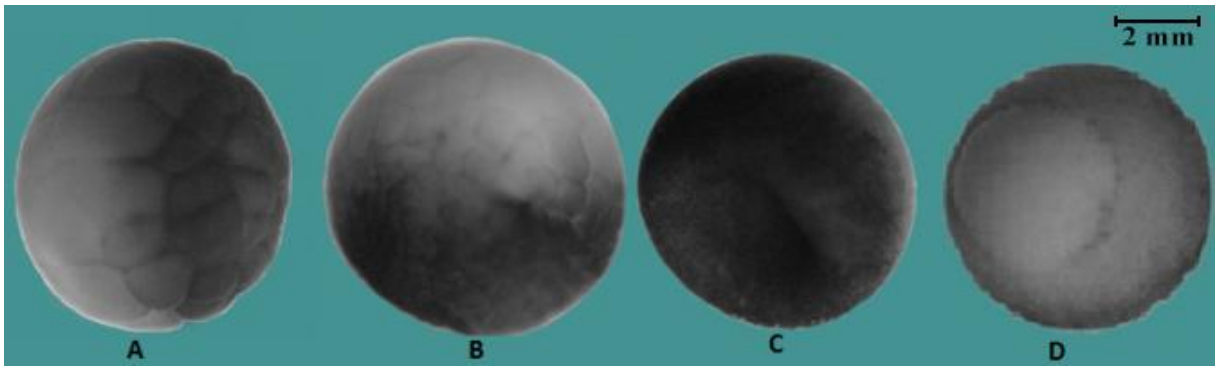

Figure 1: Some stages of embryonic development of *Pelophylax bedriagae*. (A): stage 7, 32 cells; (B): stage 9, Late cleavage; (C): stage 10, Dorsal lip; (D): stage 11, Yolk plug.

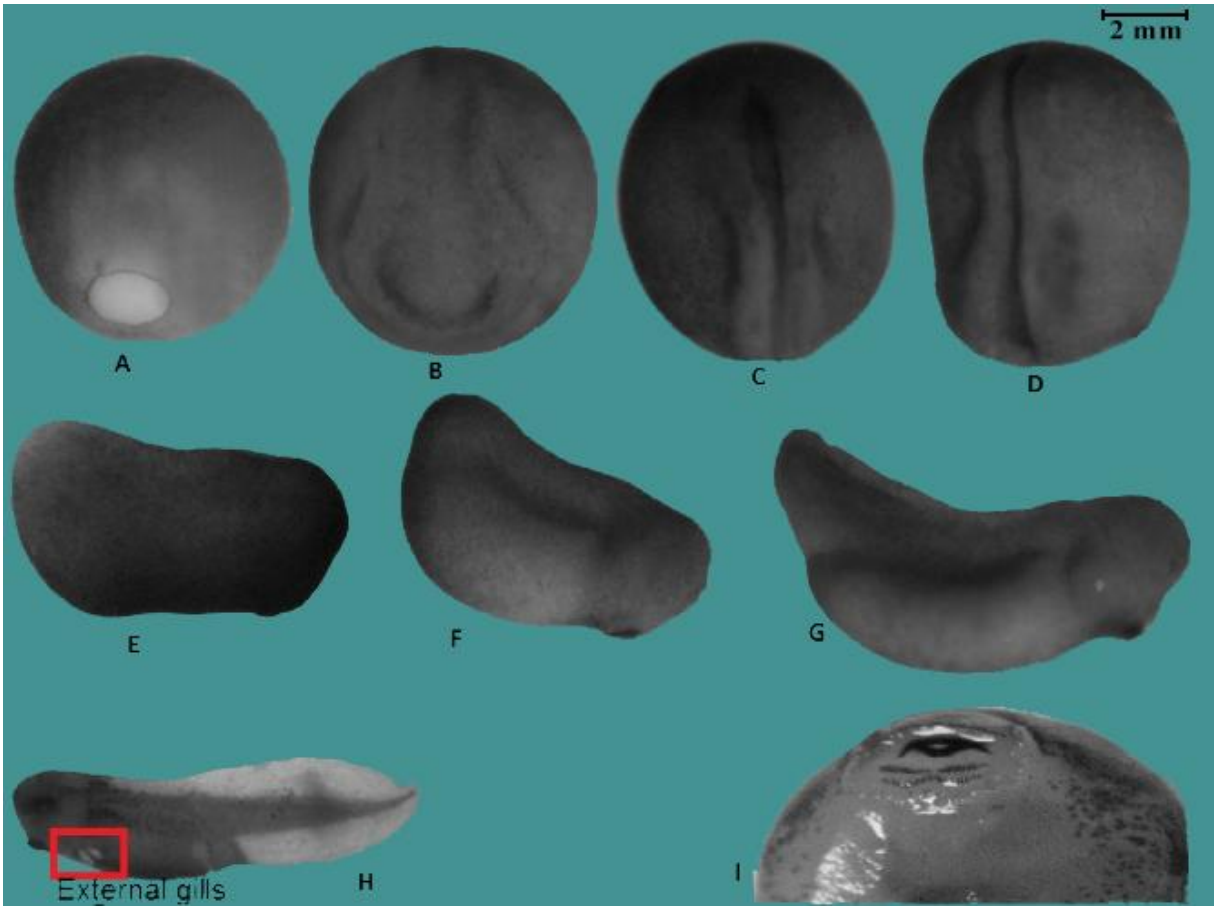

Figure 2: Some stages of embryonic development of *Pelophylax bedriagae*. (A): stage 12, Late gastrula; (B): stage 14, Neural fold; (C): stage 15, Elongation, Rotation; (D): stage 16, Neural tube, Gill plate; (E): stage 17, Tail bud; (F): stage 18, Muscular response olfactory pits; (G): stage 19, Heartbeat, Gill bud; (H): stage 20, Gill circulation, Tail elongation; (I): stage 21, Labia and Teeth differentiation.

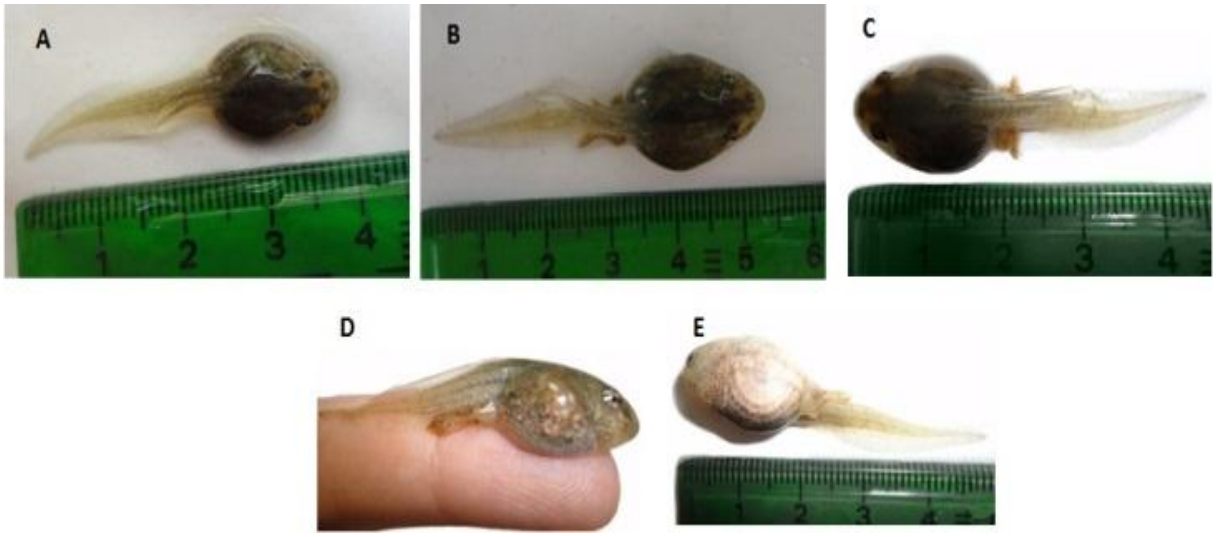

Figure 3: Some stages of larval development of *Pelophylax bedriagae*. (A-E): stages 27–40, development of hindlimbs bud.

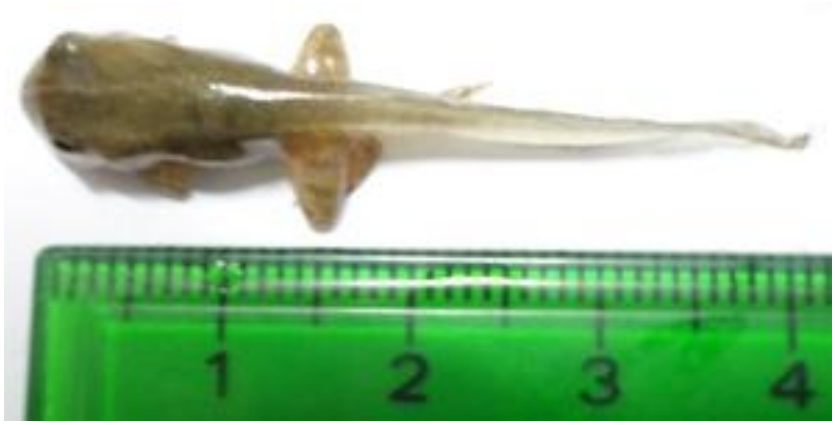

Figure 4: Stage 41 of tadpole metamorphosis in *Pelophylax bedriagae* as forelimbs are visible.

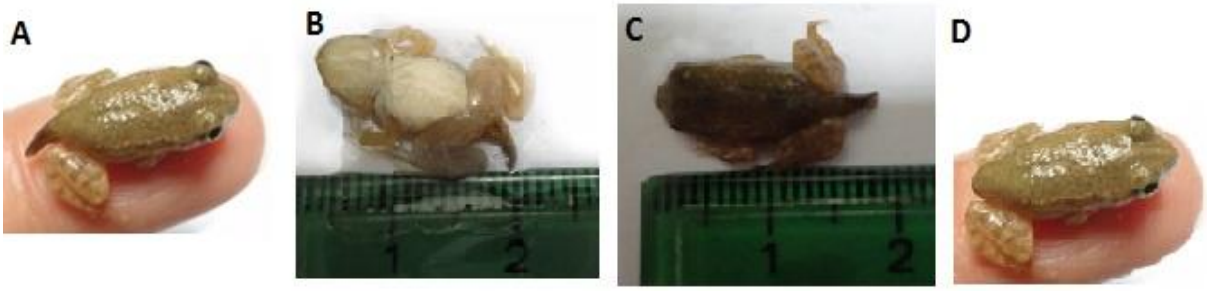

Figure 5: Stages 42–46 of metamorphosis of *Pelophylax bedriagae* (A-D).

Discussion

Amphibian behavior is the result of a complex controlling factor for many physiological processes, including temperature and humidity. For example, changes in ambient temperature could disrupt the ability to find food and their foraging efficiency, periods of hibernation and locomotion. Furthermore, the timing of breeding, developmental rate, ovulation and amplexus are associated with the ambient temperature (Donnelly and Crump, 1998; Blaustein et al., 2010).

Pelophylax bedriagae spawns in early-April in the breeding ponds in the Khorramabad region of western Iran. The sympatric amphibian species, namely *Bufotes variabilis* (Pallas) and *Hyla savignyi* Audouin are not active during this season. *Bufotes variabilis* and *H. savignyi* coexist with *P. bedriagae* and they have different breeding times.

The tadpoles of *P. bedriagae* are unique, especially with regards to their oral disc and in all stages possess a labial tooth row formula of 2(1)3/(1). The oral disc became discernible about 17 days after egg deposition at Gosner stage 21 and degenerated at Gosner stage 42. The labial tooth row formula reported in *Rana macrocnemis pseudodalmatina* (now: *Rana pseudodalmatina* Eiselt and Schmidtler) in Alang Dareh forest is 3(2)/4(1) (Ebrahimi et al., 2008). *Sylvirana nigrovittata* (Blyth) and *Hylarana erythraea* (Schlegel) from Peninsular Malaysia possess a labial tooth row formula of: 2(2)/3(1) and 1/2(1), respectively. Also, *Rana banjarana*, now in synonymy with *Pulchrana banjarana* (Leong and Lim), is the only species with a non-emarginate oral disc (Ming, 2005).

From this summary, species of the genus *Rana* exhibit a wide variation in this character. It seems that, larval length can be regulated by environmental factors, including food

availability, water temperature and other factors (Blaustein et al., 2010). The largest body length of larval *P. bedriagae* measured about 54 mm in this study but it measured 78.7 mm in the Anzali Lagoon, Iran and 125 mm in the South of France (Mirzajani et al., 2006; Momeni and Zamatkesh, 2005).

The mean number of eggs in a clutch of *Pelodytes punctatus* (Daudin), the smallest species in the genus *Pelodytes* Bonaparte is 360 (Toxopeus et al., 1993). It was also reported that the number of eggs spawned by a *Pelodytes caucasicus* Boulenger female changed with altitude, e.g., fewer eggs near sea level and more eggs in higher environments (Tuniev, 1989). Franzen (1999) reported 58–223 eggs within the clutches of *Pelodytes caucasicus*. The present study established the number of eggs spawned to be 5000–6000, depending on the female's size, a finding in accordance with the results reported by Chubinishvili et al. (1995).

In *Pelodytes punctatus*, which was reported to have rather rapid embryonic and larval development, the embryonic development until hatching took 4–14 days, depending on the ambient temperature, and metamorphosis was completed in approximately 70 days (Toxopeus et al., 1993). Also, metamorphosis was completed in 73 days for *Rana pseudodalmatina* in Golestan Province, Iran (Pesarakloo et al., 2015), 43–90 days for *P. ridibundus* in Anzali Lagoon, Iran (Mirzajani et al., 2006) and 80–120 days in France (Momeni and Zamatkesh, 2005). Whereas *P. bedriagae* eggs hatched about one week after egg deposition and tadpoles in the 24 °C treatment had finished their development and commenced metamorphosis after 111 days, some individuals in the 20 °C treatment showed a distinctly slower developmental progress which took up to 140 days.

Generally, we observed a faster growth at higher water temperatures. We cannot determine if this wide variation also takes place under natural conditions or whether this is due to the artificial environment. Development in a natural habitat may also take longer than in our study. Based on the findings of this study, it can be concluded that the development of *P. bedriagae* is slower compared to other Palearctic anurans. Such advances have the potential to improve the output of amphibian captive breeding programs and may be of value to amphibian conservation. Also, study on the developmental patterns and reproductive strategies of the other species of *Pelophylax* are highly recommended to find out if there are any similarities and differences between these species.

Studying development is important to help taxonomists assess subspecies and species more completely. We emphasis that the evolution of development is an integrated and integrative field of study, rich in empirical and heuristic value. We present our comparisons of patterns of development among diverse amphibians to help to establish a baseline for further study of species with different evolutionary and developmental histories that result in markedly different phenotypes. We anticipate that such studies will provide a more intensive and better-informed analysis of pattern and process of the evolution of development.

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